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In Vitro Drug Release Behavior of D,L-Lactide/Glycolide Copolymer (PLGA) Nanospher s with Nafarelin Acetate Prepared by a Nov l Spontan ous Emulsification Solvent Diffusion Method

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R ceived May 26, 1993, from the *Department of Pharmaceutical Engineering, Gifu Pharmaceutical University, 5-6-1, Mitahora-higashi, Gifu 502, Japan.* Accepted for publication December 13, 1993[⊗].

Abstract □ Nanospheres with D,L-lactide/glycolide copolymer (PLGA) were prepared as a biodegradable and biocompatible polymeric carrier for peptide drugs by a novel spontaneous emulsification solvent diffusion method. Nafarelin acetate (NA), a luteinizing hormone-releasing hormone analogue, was employed as a model peptide drug to investigate the encapsulation efficiency. The drug and PLGA, dissolved in an acetone–dichloromethane–water mixture, were poured into an aqueous solution of polyvinyl alcohol under moderate stirring at room temperature. Spontaneous emulsification arising from a rapid diffusion of acetone from the organic to the aqueous phase enables preparation of PLGA submicron spheres 200–300 nm in size. The entrapment of NA in nanospheres was improved by blending low molecular weight (Mw = 4500) PLGA with higher molecular weight PLGA due to the synergistic effect of th rapid deposition of PLGA and the ionic interaction between NA and PLGA. By coadmixing a small amount of negatively charged phospholipids such as dipalmitoyl phosphatidylglycerol or dicetyl phosphate, the leakage of water-soluble NA was further prevented. The NA encapsulated in PLGA nanospheres was more stable than native NA in acidic medium (pH = 1.2). The drug-release behavior from nanospheres suspended in the disintegration test solution no. 1 (Japanese Pharmacopeia XII) exhibited a biphasic pattern. It was found that the initial burst of release might be due to the degradation of the PLGA chain, as monitored by gel permeation chromatography. At a later stage, the drug was released more slowly, the rate of which was determined by the diffusion of the drug in the porous matrix structure. In the test solution no. 2 (pH = 6.8), the drug release rate from the nanospheres was much slower than that in solution no. 1.

Recently, many physiological polypeptides, e.g., autacoid, hormone, and antibody, have become easy to acquire due to the progress of biotechnological techniques. Since these molecules have significantly short biological half-lifes, repeated injections (i.e., subcutaneous, intramuscular, or intravenous) of drug over a long therapeutic period are generally required when they are used to treat disease. Prolonged-release devices composed of biodegradable and biocompatible polymers should be suitable to overcome such a practical disadvantage. The depot formulations such as injectable microcapsules with D,L-lactide/glycolide copolymer (PLGA)[1-5] and implantable small cylinders with poly-(D,L-lactide) (PLA)[6] and PLGA[7] or an alternative biodegradable polymer[8] have already been developed to extend the therapeutic effect of highly potent analogues of luteinizing hormone-releasing hormone (LH-RH). It was reported that these devices would continuously deliver the polypeptides over a period of weeks or even months at a constant rate which depended on the degradation behavior of the polymer employed.

Nonparenteral routes of administration for protein drugs, including transmucosal (nasal, ophthalmic, and pulmonary) and, particularly, oral routes, offer a number of inherent advantages over injection; they are inexpensive, easy, and convenient to administer and relative free from side effects. However, these macromolecular drugs are generally ineffective by the oral route since they are denatured by gastric acidity and the proteolytic enzymes in the gut. Even though the drugs are stable to digestion, their molecular weights are too high for absorption through the intestinal wall to occur.

Various colloidal drug carriers (i.e., liposomes or nanoparticles) might become candidates as delivery systems to improve th availability of orally administered peptide drugs and vaccines. The uptake and accumulation of inert particles into Peyer's patches, an organized lymphoid tissue on the surface of the gastrointestinal tract, has been demonstrated following chronic particle feeding.[9] Jani et al. reported that nanoparticles with diameters smaller than 500 nm, following oral administration, could cross the M-cells in Peyer's patches with retention of their intact vesicular structure, delivering the drug to the systemic circulation.[10,11] Peyer's patches are thought to play a significant role in the regulation of immune responses against antigens intestinally invaded. Therefore, studies by Eldrige and O'Hagan have shown that biodegradable PLGA or poly(alkyl cyanoacrylate) micro- or nanoparticulates could be used as adjuvants for orally administered vaccines.[12,13] Grangier et al. established the preparation method in which growth hormone releasing factor (GRF), a 44 amino acid peptide, could be associated with biodegradable poly(alkyl cyanoacrylate) nanoparticles at higher content.[14] Furthermore, oral delivery of insulin using poly-(isobutyl cyanoacrylate) nanocapsules was observed to d crease glycemia in fed diabetic rats.[15]

Nafarelin acetate, a superactive agonist of LH-RH, was loaded in the devices as a model peptide drug in order to develop a novel carrier for oral administration of this peptide. In a previous report we developed a novel spontaneous emulsification solvent diffusion method to prepare biodegradable PLGA nanospheres loaded with water-soluble and insoluble drugs.[16]

In the present work the preparation conditions w re investigated to improve the drug content in the nanospher s and the physicochemical properties, e.g., diameter, surface topography, and surface charge were characterized. Furthermore, the peptide stability and release properties of the nanospheres in imitation gut fluids were also investigated in vitro to simulate oral administration.

Experimental Section

Materials—Nafarelin acetate (NA) ([D-Nal(2)[6]]LHRH) was supplied by Syntex Research. PLGA with an average molecular weight of 12 279, 66 671, and 127 598, whose copolymer ratio of D,L-lactide to glycolide is 85:15 (abbreviated as PLGA(85-15)-12 279, PLGA(85-15)-66 671, and PLGA(85-15)-127 598, respectively), was supplied by Du Pont. PLGA-(70-30)-4500 (Taki Chemical Co., Ltd.) was purchased. The weight average molecular weight (Mw) was determined by gel permeation chromatography (GPC) by the supplier. Polyvinyl alcohol (PVA-217C, Kuraray Co., Tokyo, Japan), dipalmitoyl phosphatidyl glycerol (DPPG, Sigma Chemical Co., Ltd., St. Louis, MO), and diacetyl phosphate (DCP, Sigma Chemical Co., Ltd., St. Louis, MO) were used as supplied.

Preparation of PLGA Nanospheres—PLGA nanospheres containing nafarelin acetate were prepared by the spontaneous emulsification solvent diffusion method that was described in our previous report,[16] in which the optimized conditions were investigated to improve the recovery of nanospheres and the entrapment of water-soluble or water-insoluble

[⊗] Abstract published in *Advance ACS Abstracts*, February 15, 1994.

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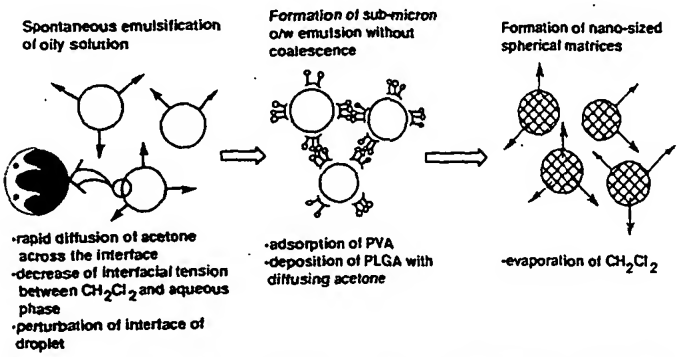

Figure 1—Preparation mechanism of PLGA nanospheres by the spontaneous emulsification solvent diffusion method.

drugs in nanospheres. Fessi et al. reported a similar preparation method for nanocapsules using interfacial polymer deposition following solvent displacement in which only water-insoluble drugs were entrapped.[17] This technique is characterized by avoiding the heat and homogenization of the system and easily scaling up to the industrial level. The mechanism for preparation of PLGA nanospheres is shown in Figure 1. PLGA (120 mg) and nafarelin acetate (3 mg) were dissolved in the mixed organic solvent of acetone (15 mL), dichloromethane (0.5 mL), and 0.2-μm-filtered water (1.5 mL). Water was admixed to dissolve NA in this organic phase. The resultant organic solution was poured into 50 mL of the aqueous PVA solution (2.0%, w/v) with magnetic stirring at room temperature. The rapid diffusion of acetone into the aqueous phase caused a remarkable decrease of the interfacial tension between the organic and aqueous phases which resulted in the organic solution forming fine droplets without strong mechanical agitation. In addition, the perturbation of the interface spontaneously produced a much larger interfacial area, which led to nanometer-sized emulsification of the organic solvent droplets dissolved the polymer. This interfacial turbulence would be governed by the well-known Marangoni effect, wherein the movement in an interface is caused by longitudinal variations of the interfacial tension.[18] The polyvinyl alcohol (PVA) molecules adsorbed on the surface of the resultant nanodroplets prevented coalescence of the fine droplets during stirring. The further diffusion of acetone and the counterdiffusion of water into droplets induced the polymer to deposit in the droplets. The emulsification system was stirred under aspirator-reduced pressure. During the 3–4-h evaporation of the dichloromethane from the droplets of mixed organic solution, the dispersed nanodroplets were solidified in the aqueous solution, forming nanosized spherical matrices. The nanosuspension, passed through a membrane filter with a 1.0-μm pore size (FR-100, Fuji Photo Film Co., Ltd.), was ultracentrifuged at 156200*g* for 1 h (CP-56G, Hitachi Koki Co., Tokyo, Japan) in order to remove the free drug that leaked out of the nanospheres and to wash out the excess PVA. The separated nanospheres were redispersed in water and the ultracentrifugation procedure was carried out again to assay the drug in the nanospheres.

Measurement of Physicochemical Properties of Nanospheres—Morphological examination of nanospheres containing nafarelin acetate was performed using a scanning (SEM, JSM-T330A, Nihon Denshi Co., Ltd.) and a transmission electron microscope (TEM, JEM-1200EX2, Nihon Denshi Co., Ltd.) following drying under cooling at 5 °C and a freeze–fracture, respectively. The ζ potential of nanospheres was measured by an electrophoretic method (Lazer Zee Meter Model 501, Pen Kem). The particle size distribution of nanospheres dispersed in the system filtered by a 1.0-μm membrane filter was measured by means of a dynamic light-scattering method (LPA-300, Otsuka Electronics Co., Ltd.). The recovery (%) of nanospheres was represented by the average ratio (of at least duplicate samples) of the weight of the result spheres passed through a filter (1.0-μm pore size) to the total weight of polymer and drug loaded. The resultant nanospheres, following sedimentation by ultracentrifugation and drying under reduced pressure, were weighed and dissolved in acetonitrile, to which a KH_2PO_4 aqueous solution (30 mM) was added to preferentially precipitate the polymer. The drug in the clear supernatant after centrifugation (10 000 rpm, 15 min, Kubota 7800, Kubota Co., Ltd.) was analyzed spectrophotometrically at 225 nm by means of a HPLC technique (pump, 880-PU; detector, 875-UV; Japan Spectroscopic Co., Ltd., Tokyo, Japan; column, Wakosil 5C8; Wako Pure Chemical Ind., Osaka, Japan). As an internal standard, hydrocortisone was employed. The drug recovery and content in the nanospheres are represented by eqs 1 and 2, respectively.

$$\text{drug recovery (\%)} = \frac{\text{the amount of drug in the nanospheres}}{\text{the amount of drug fed into the system}} \times 100 \quad (1)$$

$$\text{drug content (\%)} = \frac{\text{the amount of drug in the nanospheres}}{\text{the amount of nanospheres recovered}} \times 100 \quad (2)$$

In Vitro Release Kinetic Experiments and Stability of Nafarelin Acetate in PLGA Nanospheres—In vitro release of NA was performed by a shaking test tube procedure using an incubator (M-100, Taitec Co.) at 37 °C instead of the dialysis technique to monitor the drug released from the suspended nanospheres[16] because of the instability of the drug in solution. The nanospheres (100 mg) were suspended in 20 mL of the release media consisting of disintegration test solution no. 1 (pH = 1.2) or no. 2 (pH = 6.8) as specified in the Japanese Pharmacopeia XII (JP XII). After incubation for a suitable interval, this suspension system was neutralized at pH = 5.4 by the addition of a 1 N sodium hydroxide solution (1.8 mL) or a 10% aqueous phosphoric solution (0.25 mL) to JP XII no. 1 or no. 2 fluid, respectively, in order to protect this labile peptide from further degradation. A part of the suspension in the test tube was withdrawn to be assayed by the HPLC method mentioned above to determine the drug concentration in the whole suspension. After the residual suspension was ultracentrifuged, the NA levels in the supernatant and in the sedimented nanospheres were separately determined. The free drug (250 μg) dissolved in each release media (20 mL) was periodically analyzed using the same batch process to evaluate the stability of NA in gastrointestinal fluid.

To investigate the degradation behavior of the PLGA molecules in the nanospheres during the drug-release test, drug-free nanospheres dispersed in the release media were taken at suitable intervals to measure the molecular weight change of the PLGA released from the nanospheres, using gel permeation chromatography (GPC). The measurements were performed with a HPLC system (Japan Spectroscopic Co., Ltd., Tokyo, Japan) at 35 °C (oven, 860-CO) at a flow rate of 1.0 mL/min (pump, 880-PU) with a refractive index meter (detector, 830-RI) as detector. A 200-μL sample of the PLGA solution dissolved in tetrahydrofuran (THF) was separated with three Shodex KF-805L columns connected in series (Showa Denko Co., Ltd.). The weight average molecular weight (Mw) was calibrated with an integrator (Labchart 180, System Instruments Co., Ltd.) with the use of standard polystyrene (Shodex Standard S series).

Results and Discussion

Physicochemical Identification of PLGA Nanospheres with Nafarelin Acetate—The scanning electron microphotographs (SEM) of typical PLGA nanospheres with drug are shown in Figure 2, which indicates that nanospheres have a discrete spherical structure without aggregation. It was found that the size distribution was very sharp and ranged from 200 to 300 nm, which would be contributed to the spontaneous emulsification in the preparation procedure. PLGA microparticles have been produced by an oil-in-water (O/W) emulsion solvent evaporation method. Though the preparation conditions necessary for the production of particles below 3 μm in size were investigated by Jeffery et al.,[19] the minimum average size in that report was 1-2 μm even if the system was agitated by using a high speed homogenizer (Silverson). In the present work, spontaneous emulsification arising from the rapid diffusion of acetone across the interface would easily and reproducibily enable the preparation of PLGA submicron particles 200–300 nm in size. The surface of the nanospheres appeared rigid and film-structured and no drug crystals could be identified. The residual solvent content of the final product was less than 10 ppm, which was the measurement limit of our gas chromatograph equipped with a FID detector (GC-14A, Shimadzu Co., Ltd.).

The physicochemical properties of PLGA nanospheres are shown in Table 1. The mean diameters of the nanospheres were around 200–300 nm. Their standard deviations were rather narrow (14–31 μm), indicating monodispersed nanosph res as

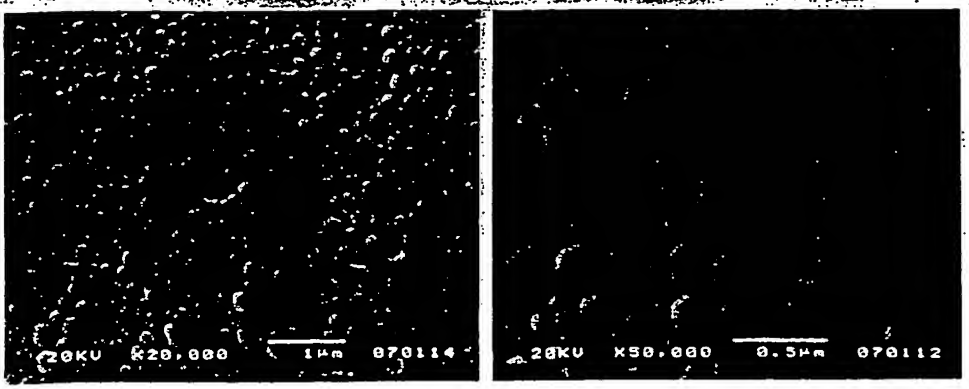

Figure 2—Scanning electron microphotographs of PLGA(85-15)-127 598 nanospheres containing nafarelin acetate.

Table 1—Entrapment of Nafarelin Acetate in PLGA Nanospheres[c] Prepared in the Dichloromethane (0.5 mL)–Acetone (15 mL)–Water (1.5 mL) System

PLGA(85-15) Mw[a]	Mean Diameter[b] ± SD (nm)	Recovery of Nanospheres (%)	Drug Recovery in Nanospheres (%)	Drug Content in Nanospheres (%)
12 279	311 ± 20	76.3	4.96	0.15
66 671	224 ± 14	79.4	11.8	0.37
127 598	233 ± 31	94.5	8.22	0.22

[a] Mw: weight average molecular weight measured by GPC. [b] Measured by photon correlation spectroscopy. [c] Polymer, 120 mg; drug, 3 mg.

observed by SEM (Figure 2). The recovery of the nanospheres increased to over 90% as the molecular weight of the PLGA increased because the higher molecular weight polymer was rapidly deposited in droplet form before it could coalesce during stirring. However, the entrapment of NA (a water-soluble peptide drug) in the nanospheres was unsuccessfully low irrespective of the molecular weight of the PLGA employed, which was attributed to the leakage of NA into the outer aqueous phase. In our previous study of the encapsulation of 5-fluorouracil, a water-soluble and low molecular weight model drug, in PLGA nanospheres, the drug recovery in the nanospheres increased as the molecular weight of the polymer employed increased.[16] It was suggested that the rapid deposition of the film-like wall at the interface between the droplets and the aqueous medium would prevent the leakage of the water-soluble drug, leading to an improvement in the trapping efficiency with increasing molecular weight. This was not the case in the present system. NA is a basic peptide compound with pK_a = 5.93 and has positively charged groups (histidyl and arginyl residues) in the molecular structure. Therefore, the amino groups of this peptide would be supposed to interact with the end carboxyl groups of the PLGA. Bodmeier et al. reported that increasing the amount of low molecular weight fractions in the polymer blend increased the number of free carboxyl groups interacting with basic compounds.[20] Furthermore, it is reported that the ionic interaction between the basic functional group of the thyrotropin releasing hormone (TRH) and the carboxylic end group of the PLGA is necessary to produce rigid microspheres without an initial burst release.[21] Therefore, the level of entrapment of NA in the present nanospheres might be determined by the ionic interaction between the drug and the polymer as well as the deposition rate of polymer at the interface.

Improvement of the Nafarelin Acetate Content in PLGA Nanospheres—Low molecular weight PLGA with Mw = 4500 was blended with three types of higher Mw PLGA (as indicated in Table 1) at various weight ratios, and the nanospheres were prepared in order to improve the encapsulation efficiency of NA (Figure 3). The addition of PLGA(70-30)-4500 caused an increase of the drug content in the nanospheres at any blend ratio, contrasting with the case of n additi n. It was assumed that the improvement of the drug recovery in the nanospheres was attained by preventing the drug leakage by the rapid precipitation

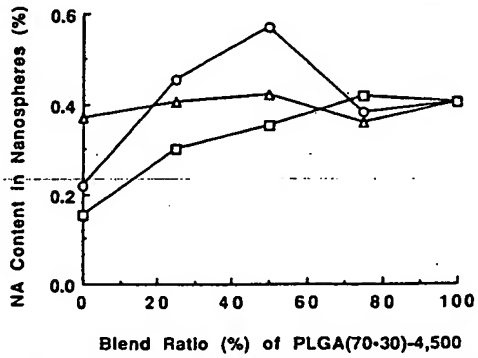

Figure 3—Effect of addition of low molecular weight PLGA on NA content in PLGA nanospheres. Key: (○) PLGA(70-30)-4500/PLGA(85-15)-127 598, (△) PLGA(70-30)-4500/PLGA(85-15)-66 671, (□) PLGA(70-30)-4500/PLGA-(85-15)-12 279. The loading amounts of blended PLGA and NA are 120 and 3 mg, respectively.

of the polymeric film due to the higher Mw PLGA and the i nic interaction between this peptide and the lower Mw PLGA. In particular, the drug content in PLGA(85-15)-127 598 nanospheres was increased remarkably and the blend ratio of 50/50 provided a maximum value of 0.57% (theoretical value = 2.44%).

Because it is expected that NA forms paired-ion bonds with negative additives, the leakage of drug into the aqueous phase can be decreased by compounding phospholipids such as dipalmitoyl phosphatidyl glycerol (DPPG) or dicetyl phosphate (DCP) which are negatively charged, lipophilic, and biocompatible. The lipophilic additives were codissolved in the oily phase with the polymer when entrapping NA into nanospheres according to the previous report.[22] Figure 4 shows the eff ct of the addition of phospholipids to the oily phase on the entrapment of the drug into nanospheres. In each phospholipid case, higher NA contents in the nanospheres were obtained with higher and lower Mw PLGA were blended at any ratio. A NA content in the nanospheres of about 1% was obtained when DPPG was added in an amount which was 3 times the molar amount of NA, but DCP had little effect on the entrapm nt of NA. The difference between DPPG and DCP was probably du to their different lipophilicities. Since DPPG had longer lipophilic groups than DCP and interacted tightly with PLGA chains,

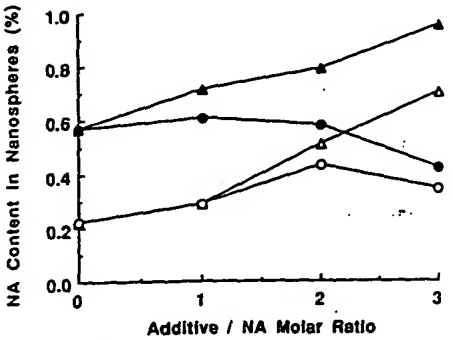

Figure 4—Effect of negatively charged additives on NA content in PLGA nanospheres with PLGA(85-15)-127 598 (O, △) or PLGA(70-30)-4500/PLGA(85-15)-127 598 = 50/50 (●, ▲). Additive: (△, ▲) DPPG, (O, ●) DCP. The loading amounts of blended PLGA and NA are 120 and 3 mg, respectively.

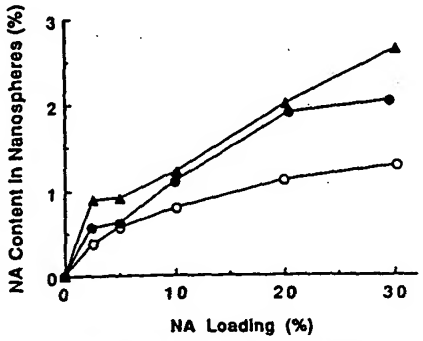

Figure 5—Effect of the loading amount of NA (weight ratio of NA to PLGA loaded) on drug content in PLGA nanospheres. Key: (O) PLGA(85-15)-66 671, ( )PLGA(70-30)-4500/PLGA(85-15)-127 598 = 50/50, (▲)PLGA-(70-30)-4500/PLGA(85-15)-127 598 = 50/50 + DPPG 4.5 mg. The loading amount of PLGA is 120 mg.

DPPG increased the partitioning of NA into the oily phase at th solvent–water interface, resulting in an improvement of the entrapping of NA into the nanospheres.

In order to increase further the drug content in the nanospheres, the loading amount of NA was increased up to a 30% level against the constant amount of polymer (120 mg). It was found that the greater the NA amount loaded, the higher the drug content in the nanospheres (Figure 5). However, the drug content in the nanospheres with PLGA(85-15)-66 671 reached only to 1.3%. When a lower Mw PLGA was blended with a higher one (blend ratio = 50/50 w/w), the drug content increased up to 2.0%. The addition of negatively charged DPPG promoted a further improvement of the NA content to a maximum value of 2.7%. These values might be very low generally but are sufficient for peptide drugs such as an LH-RH analogue like NA which has significant therapeutic activity at lower levels of administration, e.g. the microgram level.

Figure 6 shows the effect of NA loading on the drug capture into nanospheres. The retention of NA into nanospheres diminished as the loading amount of NA was increased as a result of drug leakage into the aqueous phase. Almost 80–90% of the NA could not be encapsulated into nanospheres in all cases. Whereas, Ogawa et al. reported that it was possible for leuprolide acetate, another LH-RH analogue, to be fairly well entrapped in the PLGA microcapsules with 10–20% drug content using an in-water drying method and water-in-oil-in-water emulsion.[1] In the present method, the loss of water-soluble drug was unavoidable because of th leakage that accompanied the rapid diffusion of the acetone to th aqueous phase and the significantly smaller size of the nanospheres having a larger specific surface area.

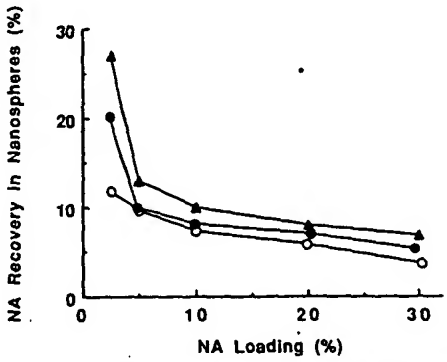

Figure 6—Effect of the loading amount of NA on drug recovery in PLGA nanospheres. Key as in Figure 5.

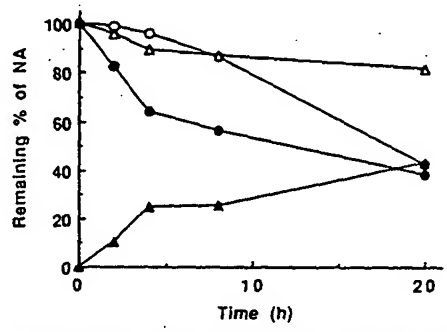

Figure 7—Release profiles of NA from PLGA(85-15)-66 671 nanospheres and stabilization of NA by nanoencapsulation during the release test in JP XII no. 1 fluid. Key: (O) NA solution, (△) nanosuspension, ( )nanospheres, (▲) supernatant.

Release and Stabilization Behavior of Nafarelin Ac tate in PLGA Nanospheres in Vitro—The degradation and the release behavior of NA in PLGA nanospheres were investigated in the JP XII no. 1 and no. 2 fluids to simulate the behavior of nanospheres exposed in the gastrointestinal tract following oral administration. The nanospheres prepared with a 5% NA loading to PLGA(85-15)-66 671 were used as a sample for the release test. PLGA(70-30)-4500 has a glass transition temperature of 32 °C, so the nanospheres composed of PLGA(85-15)-127 598/PLGA(70-30)-4500 were not used as a sample for the release test at 37 °C because they might be likely to soften and aggregate in the test tube. In general, GET (gastric emptying time) values for the solid dosage forms such as tablets or granules were approximately <1 h under fasting and 2–3 h under feeding, respectively, and their small intestinal transit time was constantly 3–4 h under any condition. However, the duration of the release test was prolonged for 20 h since colloidal particles such as the present nanospheres might be adsorbed or creep into the microvilli or the glycocalyx, which is a uniform layer of filamentous glycoprotein in the digestive lumen.[23] It was found that the colloidal suspension of PLGA nanospheres was physically stable against aggregation or coalescence during the release test because of their negative ζ potentials [−7.0 and −16.3 mV in JP XII no. 1 (ionic strength = 0.0975) and no. 2 solutions (0.3236), respectively].

Native NA in aqueous solution is reasonably stable at pH = 4–6 (most stable at pH = 5.4) but labile in acidic and alkaline solution.[24] In the present test in the acidic fluid (no. 1), more than 55% of native NA dissolved was degraded during incubation for 20 h at 37 °C, whereas more than 80% of the NA remained unchanged in the nanosuspension (Figure 7). This result suggests that the stability of NA, which is a peptide drug, in the stomach might be much improved by it being entrapped into nanospheres. The release behavior, which is represented by the amount of NA either dissolved in the supernatant or remaining in the nanos-

Figure 8—Transmission electron microphotograph of freeze-fractured PLGA nanospheres containing nafarelin acetate. PLGA(70-30)-4500/PLGA-(85-15)-127 598 = 50/50.

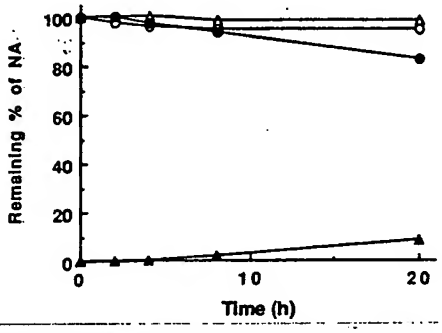

Figure 9—Release profiles of NA from PLGA(85-15)-66 671 nanospheres and stabilization of NA by nanoencapsulation during the release test in JP XII no. 2 fluid. Key as in Figure 7.

pheres, indicated a biphasic pattern. The initial burst stage, during which over 25% of the NA loaded in the nanospheres was rapidly released over 4 h, was followed later by a sustained-release stage. The larger specific surface area nanospheres compared with the micron-sized particles promoted the burst release at the initial stage. It was reported that the nanoparticles following oral administration could reach and cross the M-cells of Peyer's patches located in the jejunum and particularly the ileum.[10,11] Therefore, free NA that was rapidly released from the nanospheres at the burst stage in acidic solution (JP XII no. 1), as in the stomach before reaching Peyer's patches, was assumed to be pharmacologically ineffective due to its denaturation and nonpermeability across the gastrointestinal wall. The nanospheres must be further elaborated to decrease the initial burst amount during the release test in JP XII no. 1 fluid as much as possible.

The transmission electron microphotograph, prepared by the freeze-fracture method, showed that the PLGA nanosphere had a porous matrix structure instead of a capsule structure (Figure 8). The pores observed in the nanospheres were assumed to work as a diffusive water path, through which the drug diffused out slowly, leading to the sustained-release period at the later stage.

In the dissolution test solution no. 2 (JP XII), native and encapsulated NA were reasonably stable and the drug-rel ase rate from the nanospheres was much slower than that in test solution no. 1, without a burst of drug release as shown in Figure 9. The difference of release behavior in fluid no. 1 might be attributed to the stability of PLGA as well as native NA. Therefore, the degradation of the polym r was monitored by measuring th molecular weight of the polymer composing the

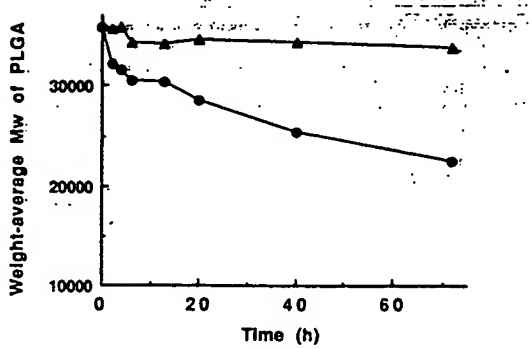

Figure 10—Degradation profiles of PLGA(85-15)-66 671 nanospheres during the release test. Test fluid: (●) JP XII no. 1, (▲) JP XII no. 2.

nanospheres with gel permeation chromatography (Figur 10). It was found that the burst of NA release in fluid no. 1 was attributed to the degradation of the polymer. The molecular weight of PLGA gradually decreased in acidic solution from the initial stage. It was reported that the weight loss of PLGA microcapsules, i.e. hydrolysis of the PLGA chain, started after a lag time depending on the molecular weight and the copolymer ratio at the subcutaneously injection site in rats.[3] However, the lag time of Mw loss of PLGA nanospheres might disappear in the present system because of their larger specific surfac area compared with that of microcapsules. On the other hand, the degradation of the polymer did not substantially occur in fluid no. 2. This result also explained the correlation between the drug-release behavior and the degradation profile of PLGA.

In conclusion, a peptide drug (nafarelin acetate) was successfully encapsulated in PLGA nanospheres by means of the novel spontaneous emulsification solvent diffusion method using an O/W emulsion system. By coadmixing acetone into the dispersed organic phase, the diameter of the nanospheres was spontaneously reduced to 200–300 nm without vigorous homogenization due to the rapid diffusion of acetone across the interface into the aqueous medium. During evaporati n of the organic solvent from the system, the polymer and the drug were coprecipitated in the dispersed nanodroplets, forming PLGA nanospheres. The entrapment of NA in the nanospheres was improved by blending lower molecular weight PLGA or negatively charged additives such as DPPG or DCP due to th ionic interaction between the basic amino groups of NA and the carboxylic or phosphatic groups of the additives. However, the loss of NA was still high (over 70%) as a result of drug leakage to the aqueous phase. Further investigations are necessary to improve the encapsulation efficiency of water-soluble peptides in PLGA nanospheres. The drug in the nanospheres was stabilized in the acidic medium compared with the native drug due to the protection against hydrolysis. The drug was rel ased from nanospheres in acidic medium, following the biphasic pattern of initial burst and sustained release. Because the initial burst release should be avoided in the case of oral administration, the nanospheres must be further designed to reduce th burst amount.

Further in vivo studies on the uptake and translocation properties through the Peyer's patches and on drug absorption after oral administration of the PLGA nanospheres with NA should be investigated to clarify the possibility of their use as carriers of the peptide. Furthermore, the present nanospheres are attractive for parenteral (intramuscular, subcutaneous, and intravenous) or transmucosal (nasal, pulmonary, and ophthalmic) application because of their discrete submicr n-sized structure and their biodegradability.

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Acknowledgments

The supplies of NA and PLGA from Syntex Japan and Du Pont are sincerely acknowledged. We thank Mr. M. Sato at Sanwa Chemical Co., Ltd., and Dr. A. Schade for the use of the Zeta Meter and useful advice in the preparation of manuscript, respectively. The authors are grateful to Mr. Akihiro Tamada for his technical assistance.